

University of Groningen

Play is indispensable for an adequate development of coping with social challenges in the rat

Hol, T.; van Ree, J.M.; Spruijt, B.M.; Everts, H.; Koolhaas, J.M.

Published in:
Developmental Psychobiology

DOI:
[10.1002/\(SICI\)1098-2302\(199903\)34:2<129::AID-DEV6>3.0.CO;2-L](https://doi.org/10.1002/(SICI)1098-2302(199903)34:2<129::AID-DEV6>3.0.CO;2-L)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
1999

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Hol, T., van Ree, J. M., Spruijt, B. M., Everts, H., & Koolhaas, J. M. (1999). Play is indispensable for an adequate development of coping with social challenges in the rat. *Developmental Psychobiology*, 34(2), 129-138. [https://doi.org/10.1002/\(SICI\)1098-2302\(199903\)34:2<129::AID-DEV6>3.0.CO;2-L](https://doi.org/10.1002/(SICI)1098-2302(199903)34:2<129::AID-DEV6>3.0.CO;2-L)

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

C. L. van den Berg

T. Hol

J. M. Van Ree

B. M. Spruijt

Rudolf Magnus Institute for
Neurosciences

Utrecht University

P. O. Box 80040, 3508 TA Utrecht
The Netherlands

H. Everts

J. M. Koolhaas

Department of Animal Physiology
University of Groningen

P. O. Box 14, 9750 AA Haren
The Netherlands

Play is Indispensable for an Adequate Development of Coping with Social Challenges in the Rat

Received 17 February 1998; accepted 6 July 1998

ABSTRACT: In this study, young rats were deprived of early social interactions during weeks 4 and 5 of life. Different behavioral tests were conducted in adulthood to study the behavioral responses of rats lacking early social experiences. Juvenile deprivation resulted in decreased social activity and an altered sexual pattern, but did not affect locomotor activity or the performance in the elevated plus maze. Furthermore, behavioral and neuroendocrine responses of juvenile isolated rats were dramatically altered when they were confronted with territorial aggression. Juvenile deprived rats did not readily display a submissive posture in response to the resident and showed no immobility behavior after being returned to the resident's territory, while their plasma corticosterone and adrenaline concentrations were significantly increased compared to nonisolated controls. In contrast, behavioral responses in the shock prod test were not affected by previous isolation. The results suggest that early social experiences are vital for interactions with conspecifics later in life, i.e., aggression, sexual, and social interactions. © 1999 John Wiley & Sons, Inc. *Dev Psychobiol* 34: 129–138, 1999

Keywords: juvenile isolation; social play; social interactions; neuroendocrine response; territorial aggression

The occurrence of social play in a variety of species has long been recognized (Groos, 1898; Small, 1899) as relevant for the development of adult social behaviors (Bekoff, 1974; Scott & Marston, 1950; Taylor, 1980). Social play is composed of behavioral patterns

related to adult social, sexual, and agonistic behavior, although juvenile and adult social behavior differ both in intensity, form and contextual settings (Panksepp, Siviy, & Normansell, 1984; Poole & Fish, 1976; Taylor, 1980). Scott and Marston (1950) concluded that environmental manipulations are most effective in periods when new social relationships are being developed. Because social play can be considered as the first extensive social contact with animals other than the

Correspondence to: B. M. Spruijt
Contract grant sponsor: Korczak Foundation for Autism and Related Disorders

mother, it is not surprising that rearing rats without the opportunity to play results in disturbed social (Meaney & Stewart, 1979), agonistic (Lore & Flannelly, 1977) and sexual behavior (Gerall, 1963; Gerall, Ward & Gerall, 1967; Gruendel & Arnold, 1969; Härd & Larsson, 1968). Panksepp and co-workers (1984) found that short-term social isolation reflects play deprivation and not social deprivation, *per se*: Housing with an older rat (which hardly exhibits play behavior) does not reduce play motivation while physical contact with young rats does. The specific nature of social play is characterized by its rewarding properties and the involvement of substrates associated with reward: opioid and dopaminergic systems (Humphreys & Einon, 1981; Niesink & Van Ree, 1989; Panksepp et al., 1984). Naturally rewarding behaviors such as feeding, drinking, and sexual behavior are important for the survival of the individual and the species. By analogy, it is assumed that social play most likely also fulfills a primary function. Social play may be crucial for understanding intraspecific communication or learning to use certain behavioral patterns in appropriate contexts (Lore & Flannelly, 1977; Meaney & Stewart, 1981). Social play behavior is predominant during a relatively short period of time, starting around Day 18 of life and declines with the approach of sexual maturation. The key element of social play, termed *pinning*, follows an inverted U-shaped distribution during development with its peak between 30–40 days of age (Baenninger, 1967; Bolles & Woods, 1964; Meaney & Stewart, 1981; Panksepp et al., 1984; Small, 1899). As social play is probably necessary for normal development of adult social behavior and social adaptive capacities, the aim of the present study is to investigate how play-deprived rats cope with social and nonsocial challenges. Nonsocial situations included open-field exposure, performance on the elevated plus maze and shock prod test. Coping with aggression (a resident intruder paradigm), sexual and social situations were studied as social challenges. Furthermore, we measured the neuroendocrine responses to territorial aggression, a form of social stress.

METHODS

Animals and Rearing Conditions

Male Wistar rats (GDL, Utrecht, The Netherlands) were used in each of the experiments. After weaning at the age of 21 days, rats were housed either individually (isolated, $n = 10$ rats) or in social groups (non-isolated, $n = 10$ rats) of 5 per cage ($40 \times 26 \times 20$ cm) from Day 22 to Day 35. After Day 35 all rats were rehoused in pairs with a rat of the same treatment

group. All rats were kept on a reversed photoperiod, lights on at 1900 hr and off at 0700 hr in a temperature-controlled room ($21 \pm 1^\circ\text{C}$). For each behavioral test a different group of 20 rats was used, except for the social defeat experiment. In this experiment 16 male Wistar rats (TNO, Zeist, The Netherlands), weaned at 21 days of age, were used. The deprivation consisted of individual housing in macrolon cages ($30 \times 25 \times 15$) from Day 22 to Day 35. At Day 35 they were resocialized together with other isolated rats in groups of 5 rats per macrolon cage ($40 \times 60 \times 15$). Control rats were group-housed (5 rats) in similar cages. Food and water were available *ad libitum*. All experimental rats were transported to the test rooms at least 2 hr before the behavioral experiment started and all tests were performed between 1100 hr and 1500 hr. Experimental procedures were approved by the Committee on Animal Experiments of the Faculty of Medicine, Utrecht University.

Social Interaction Test

The social interaction test took place when the rats had reached the age of 6 weeks. The test arena was a plastic cage measuring $70 \times 70 \times 50$ cm placed in a soundproof room. The test cage was illuminated by two 60-W red light bulbs placed 60 cm above the test cage as described by Vanderschuren, Stein, Wiegant, and Van Ree (1995). Behavioral testing consisted of placing 1 rat that had been isolated during Weeks 4 and 5 and 1 nonisolated control rat together in the test cage. All experimental sessions were recorded on videotape and lasted 10 min. Analysis from the videotape recordings was performed following behavioral testing. The durations and frequencies of the following behaviors were scored: *approaching/following* (moving in the direction of the test partner), *social exploration* (grooming or sniffing the body of the test partner), *anogenital sniffing* (sniffing the anogenital area of the test partner), and *nonsocial behaviors* (self-grooming, rearing, and exploration of the cage).

Open-Field Test

The test arena was a circular, black open field (diameter 140 cm) placed in a soundproof room under low-light conditions. The rats were observed for 10 min by a fully automated observation system (Etho-Vision, Noldus Information Technology b.v., Wageningen, The Netherlands). An object (lead-filled glass jar) was placed in the middle of the open field and at the beginning of a session each rat was placed in the outer zone of the arena at the same location. The traveled distance (in cm) was calculated as described by

Spruijt, Josephy, Van Rijzingen, and Maaswinkel (1994). The open-field test took place when the rats had reached the age of 10 weeks.

Elevated Plus-Maze

Rats were exposed to the elevated plus-maze (EPM) at 11 weeks of age. The apparatus consisted of a central 30×30 cm platform, two 40×10 cm open arms and two $40 \times 10 \times 22.5$ cm closed arms about 70 cm from the floor. A 40-W lamp illuminated the central platform at about 30 cm above the center. Testing took place in a soundproof room under low-light conditions. At the beginning of the 5-min session, the rat was placed on the central platform and the time spent on the platform, open and closed arms, and the number of visits in each arm was scored directly by an observer sitting outside the test room using a keyboard data acquisition program (Observer, Noldus Information Technology b.v., Wageningen, The Netherlands).

Shock-Prod Burying Task

Rats were exposed to the shock prod test at 11 weeks of age. The experiment was carried out in a $30 \times 30 \times 40$ cm transparent box with a metal bottom covered with sawdust which was replaced between each trial. Through a small hole in the center of the front wall of the cage, 2 cm above the bedding material, the shock prod was inserted. This shock prod consisted of a teflon prod (length: 6.5 cm; ϕ : 1.0 cm) with two uninsulated wires (ϕ : 0.5 mm) independently wrapped around it. When both wires were touched simultaneously by the rat, the animal received a 2 mA shock (Diamant, Croiset, De Zwart & De Wied, 1991). Data collection started after the first shock and the rats were observed for 10 min. During the whole test period the shock circuit was left on. Time spent on *immobility* behavior (freezing, no motion for at least 2 s), *self-grooming*, *burying* (moving bedding material to and over the prod), and *exploration* (rearing and exploration in the test cage) was scored directly by an observer using a keyboard data acquisition program (Observer, Noldus Information Technology b.v., Wageningen, The Netherlands).

The Resident–Intruder Paradigm

At the age of 10 weeks all animals were provided with a silicon heart catheter. A cannula (i.d. 0.5 mm; o.d. 1.0 mm) was placed into the entrance of the right atrium (vena cava) via the jugular vein, under complete ether anaesthesia. This method allows frequent blood sampling during a long period without disturb-

ing the behavior or the physiology of the animal (Steffens, 1969). The rats were allowed to recover after surgery for 2 weeks. During this period the rats were habituated to the blood sampling procedure by connecting them every day to a blood sampling tube.

For the resident–intruder paradigm (a variation on the procedure described by Koolhaas et al., 1990) Tryon Maze-Dull S3 rats (TMD-S3) were used. These rats were kept individually in a large ($40 \times 40 \times 80$ cm) cage together with a sterilized female. Several months before the present study, the TMD-S3 rats were repeatedly confronted in their home cage with younger male intruders to ensure a reliable level of dominance over the intruding experimental animals. After removal of the sterilized female, the experimental rat (12 weeks of age) was placed into the home cage of the resident rat. The experimental rat was in all cases attacked immediately and after 5 min the dominant TMD-S3 rat was confined in a small wire-mesh cage in its territory to prevent further attacks. The behavior of the experimental rat during and after the social defeat was observed. Time until submissive posture (lying motionless on the back for at least 5 s) was registered and after confinement of the dominant *immobility behavior* (motionless for at least 2 s), *self-grooming* and *exploration* of the intruders were scored. Blood samples (0.45 ml) were taken before (at $t = -10$ and $t = -1$ min) and after the social defeat ($t = 5$ min). After the dominant had been confined in the wire-mesh cage another blood sample was taken ($t = 15$ min). After 10 min the experimental rat was removed from the resident's territory and placed back in its own cage, and two more blood samples (at $t = 30$ and 60 min) were taken.

Sexual Behavior

Stimulus female Wistar rats (200 g at the beginning of the experiment, $n = 10$) were bilaterally ovariectomized through two lumbar incisions under a combination of Hypnorm® (Janssen Pharmaceutica, Beerse, Belgium; 0.05 ml/100 g, i.m.) and Dormicum® (Hoffman-LaRoche, Mijdrecht, The Netherlands; 0.1 ml/100 g, i.p) narcosis. The operated female rats were given at least 2 weeks to recover before the experiments started and they were housed under similar conditions as the male Wistar rats described above. Female rats were made sexually receptive and proceptive by sequential treatment with Dimenformon prolongatum (oestradiolbenzoate and oestradiolphenylpropionate, Organon B.V., Oss, The Netherlands) in a concentration of 10 μ g oestradiolbenzoate in 0.1 ml oil/rat subcutaneously, 48 hr before testing, and Progesterone (Organon B.V., Oss, The Netherlands) in a concentration of 250 μ g in 0.1 ml oil/rat subcu-

taneously 3 to 4 hr before testing. Sexual behavior testing of sexually naive rats took place at 13 weeks. To accustom the males to the test arena (plastic cage measuring $49 \times 30 \times 30$ with sawdust), they were placed in the new environment for 15 min before the receptive female was introduced. The following behavioral parameters of the male rat were scored directly by an observer sitting in the experimental room during a 20-min test using a keyboard data acquisition program (Observer, Noldus Information Technology b.v., Wageningen, The Netherlands): *mounting*, *intromission*, *ejaculation*, *anogenital sniffing* (sniffing the anogenital area of the female), *social sniffing/grooming* (grooming or sniffing the body of the female), and *self-grooming*.

Assessment of Hormonal Levels

All blood samples were instantly transferred to chilled centrifuge tubes (0°C) containing 0.01% EDTA as antioxidant and 10 μ l heparin solution (500 IU/ml) as anticoagulant. Blood was centrifuged at 4°C for 10 min at 5000 rpm. One hundred μ l of the supernatant were stored at -20°C for later corticosterone (CORT) determination and 100 μ l at -80°C for catecholamine [noradrenaline (NA) and adrenaline (A)] measurements. For plasma CORT assessments, a reversed-phase, high-performance liquid chromatography (HPLC) technique was used as described in Dawson, Kontur, & Monjan (1984). NA and A were extracted from the samples by using an extraction system as described in Smedes, Kraak, & Poppe (1982). After extraction, the NA and A concentrations were determined by means of HPLC in combination with electrochemical detection (ECD) as reported in detail by Korte and colleagues (Korte, Smit, Bouws, Koolhaas, & Bohus, 1990).

Statistics

The results of the behavioral responses in the social interaction test, the open field, elevated plus maze, shock prod burying task, social defeat, and sexual behavior are expressed as means \pm SEM. Behavioral measurements were subjected to Student *t* tests to calculate statistical significance between isolated and nonisolated rats using SPSS for Windows software, Version 6.0. Neuroendocrine parameters were evaluated by means of an analysis of variance with repeated measurements. A subsequent post-hoc Tukey *t* test was used to determine the source of significance between isolated and nonisolated rats. For statistical calculations of the neuroendocrine data, the statistical

package of SYSTAT (Wilkinson, Leland, SYSTAT: The System for Statistics. Evanston, IL: SYSTAT, Inc., 1990) was used.

RESULTS

Social Behavior

Rats isolated in Weeks 4 and 5 of life were tested versus nonisolated control rats. In a 10-min session a decrease in the number of anogenital sniffing bouts, $t = 2.2$, $df = 18$, $p < 0.05$, and approaches to the test partner, $t = 2.1$, $df = 18$, $p < 0.05$, was observed in the isolated rats as compared to nonisolated controls. No differences were observed in social exploration and nonsocial behaviors as shown in Figure 1. Furthermore, both the bout length, $t = 2.2$, $df = 18$, $p < 0.05$ (isolated: 2.8 ± 0.4 , nonisolated: 4.3 ± 0.6), and total time, $t = 2.5$, $df = 18$, $p < 0.05$ (isolated: 21.9 ± 7.3 , nonisolated: 51.9 ± 9.4), of approaching was decreased.

Open Field

Juvenile isolation during Weeks 4 and 5 of life did not affect locomotor activity when tested in adulthood in an open field. The traveled distance (in cm) of previously isolated rats (6387.8 ± 306.4) was not different from nonisolated control rats (5947.8 ± 185.7).

Elevated Plus-Maze

Isolation during Weeks 4 and 5 of life did not result in differences on the total time spent on open and closed arms in the elevated plus maze as compared to nonisolated control rats as shown in Table 1. Furthermore, the number of crossings, i.e., total number of visits, was not different between both groups (16.8 ± 1.6 for isolated rats versus 15.1 ± 1.6 for nonisolated control rats).

Shock Prod Burying Task

Both groups received several shocks upon contact with the electrified prod, but no significant differences were observed between the two experimental groups (isolated rats: 4.4 ± 0.5 shocks; nonisolated controls: 4.7 ± 0.5 shocks during a 10-min session). During the observation period both groups displayed immobility, exploration, grooming, and burying (Table 2), but no differences were observed in the behavioral responses during the session.

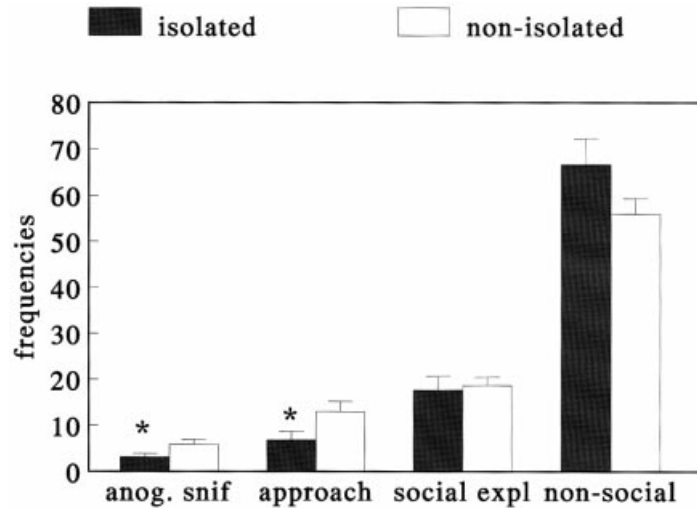


FIGURE 1 Effects of juvenile isolation (during Weeks 4 and 5 of age) on social behavior in rats tested in Week 6 of life during a 10-min session ($n = 10$ rats/housing condition). Data are expressed as mean number \pm SEM and $*p < 0.05$ (Student t test). Anog. Snif = anogenital sniffing of the test partner, approach = approach/ following of the test partner, social expl = social sniffing and grooming of the test partner and nonsocial = self-grooming, rearing and exploration of the test cage.

Social Defeat

Behavioral Parameters. Isolation in Weeks 4 and 5 of life caused a significant increase in submission latency in adulthood after being placed in a territory of a dominant rat and promptly being attacked, $t = 2.29$, $df = 10$, $p < 0.05$. This difference in submission latency cannot be attributed to a difference in attack latency by the dominant because the attack latency was not different between the isolated and nonisolated controls (Table 3). As a result of clogged cannulas, some animals were excluded from this experiment.

In Figure 2 the behavioral parameters of the rats after confinement of the resident are depicted. Non-

isolated rats displayed immobility behavior for almost the entire observation period. Rats that had been isolated showed a significantly different behavioral pattern. They started to explore the cage, $t = 12.2$, $df = 10$, $p < 0.001$, spent more time on grooming, and hardly displayed immobility behavior, $t = 8.3$, $df = 10$, $p < 0.001$.

Neuroendocrine Parameters. Baseline levels of all three hormonal parameters were measured in the 10-min period preceding the attack and were not significantly different between the isolated and nonisolated controls.

Table 1. Effects of Juvenile Isolation (during Weeks 4 and 5 of Life) on the Performance in the Elevated Plus Maze (EPM) at 11 Weeks of Age

	Nonisolated Rats		Isolated Rats	
Time open arms (seconds)	15.5 \pm 4.3		19.9 \pm 5.6	
Time closed arms (seconds)	193.4 \pm 13.4		193.0 \pm 10.8	
Time platform (seconds)	84.2 \pm 9.6		84.9 \pm 9.6	

Note. No differences were observed between isolated and non-isolated controls ($n = 10$) on time spent (seconds) on the platform, and the open and closed arms of the EPM in a 5-min test (Student t -test). Data are presented as mean duration in seconds \pm SEM.

Table 2. Effects of Juvenile Isolation (during Weeks 4 and 5 of Life) on a Shock Prod Burying Task at 11 Weeks of Age

	Nonisolated Rats	Isolated Rats
Grooming (% of total time)	0.4 \pm 0.2	1.6 \pm 0.7
Exploration (% of total time)	65.4 \pm 4.6	58.2 \pm 3.2
Immobility (% of total time)	2.0 \pm 0.9	5.9 \pm 2.8
Burying (% of total time)	31.4 \pm 4.8	33.8 \pm 3.9

Note. Data are presented as mean percentage of total time \pm SEM. A Student t test revealed no differences between the isolated and nonisolated controls ($n = 10$) on time spent on grooming, exploration, immobility, and burying.

Table 3. Attack Latencies and Submission Latencies of Nonisolated ($n = 5$) and Isolated Rats (Isolation during Weeks 4 and 5 of age) ($n = 7$) during Agonistic Encounters with a Dominant, Territorial TMD S3 Rat at 12 Weeks of Age

	Nonisolated Rats	Isolated Rats
Attack latency (seconds)	22.2 \pm 9.1	26.6 \pm 13.0
Submission latency (seconds)	142.2 \pm 18.2	237.7 \pm 32.5*

Note. Data are presented as mean latency in seconds \pm SEM and * $p < 0.05$ versus nonisolated control rats (Student t test).

Corticosterone. An ANOVA on the CORT plasma values per group revealed a significant increase after introduction of the intruder in both groups, nonisolated: $F(5, 20) = 7.1$, $p < 0.005$; isolated rats: $F(5, 25) = 37.7$, $p < 0.001$ (Figure 3a). An ANOVA with repeated measurements revealed a significant group by time interaction between the two experimental groups, $F(5, 45) = 2.5$, $p < 0.05$. This difference was caused by the more rapid decline of CORT plasma levels in nonisolated rats; at $t = 60$ these values were not different from baseline levels, $t = -10$ and -1 .

Adrenaline. Social defeat caused an increase in plasma adrenaline levels in nonisolated and isolated

rats as shown in Figure 3b, nonisolated controls: $F(5, 20) = 3.9$, $p < 0.05$; isolated rats: $F(5, 30) = 6.2$, $p < 0.001$. An ANOVA revealed a significant effect of isolation, $F(1, 10) = 5.22$, $p < 0.05$. A significant Group \times Time interaction was observed, $F(5, 50) = 2.88$, $p < 0.05$. A post-hoc Tukey test revealed that this was caused by significantly increased A levels in isolated rats just after the social defeat had taken place, $t = 5$ min.

Noradrenaline. As shown in Figure 3c, plasma NA levels of both isolated and nonisolated rats increased after social defeat, nonisolated: $F(5, 20) = 10.7$, $p < 0.001$; isolated rats: $F(5, 30) = 5.44$, $p < 0.005$. An ANOVA revealed no significant difference between the experimental groups, $F(1, 10) = 1.93$.

Sexual Behavior

Juvenile isolation did not influence the capacity to perform sexual motor acts; the number of mounts, intromissions, and ejaculations did not differ between isolated and nonisolated rats as shown in Table 4. However, in play-deprived rats, the socio-sexual behavior was affected. The number of times anogenital sniffing occurred was decreased in isolates as compared to nonisolated controls, $t = 2.1$, $df = 18$, $p < 0.05$; 2.8 ± 1.2 for isolates versus 9.1 ± 2.7 for the nonisolated controls, and the latency until the first anogenital investigation is significantly increased in isolated

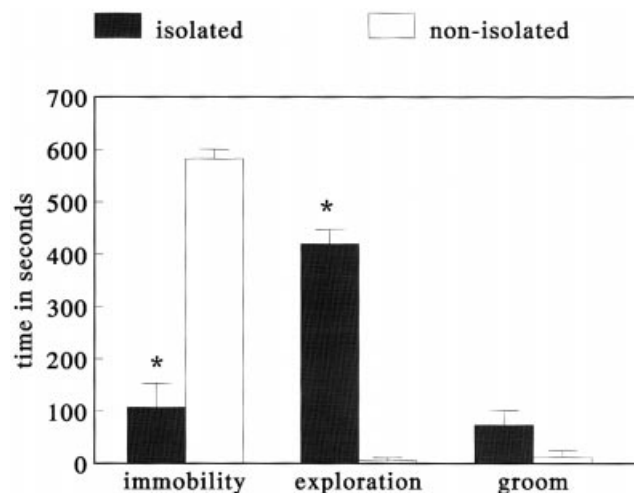


FIGURE 2 Effects of juvenile isolation (during Weeks 4 and 5 of life) on the behavioral responses to territorial aggression during a 10-min session after a social defeat at 12 weeks of age. The dominant, aggressive rat was present, but confined in a small wired cage. Data are expressed as mean duration in seconds \pm SEM and * $p < 0.05$ (Student t test). Immobility behavior (motionless for at least 2 s), groom = self-grooming and exploration of the intruder were scored.

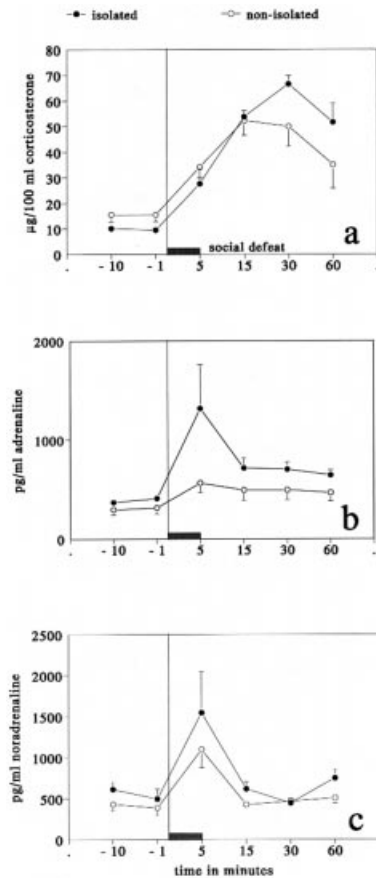


FIGURE 3 Effects of juvenile isolation (during Weeks 4 and 5) on the (a) corticosterone (CORT), (b) adrenaline (A), and (c) noradrenaline (NA) levels in response to territorial aggression at 12 weeks of age. Samples were taken 10 and 1 min before the social defeat (basal levels; -10 and -1) and after the defeat at 5, 15, 30, and 60 min. Data are expressed as mean \pm SEM.

rats, $t = 2.7$, $df = 18$, $p < 0.05$; isolated: 778.3 ± 155.9 ; nonisolated: 256.8 ± 113.2) as compared to nonisolated controls.

DISCUSSION

In the present study, the behavioral responses in nonsocial and social situations of rats lacking early social experiences were investigated. In nonsocial tests (open-field, EPM, and shock prod burying task) the juvenile isolated rats did not differ from nonisolated control rats, although several reports describe isolation-induced hyperactivity in an open field (Einon & Morgan, 1978; Einon, Morgan, & Kibbler, 1978; Robbins, Jones, & Wilkinson, 1996) and an abnormal per-

Table 4. Effects of Juvenile Isolation (during Weeks 4 and 5 of Life) on the First Sexual Experience at 13 Weeks of Age

	Nonisolated Rats	Isolated Rats
Mount	16.9 ± 6.0	14.2 ± 2.0
Intromission	6.0 ± 2.2	6.1 ± 2.2
Ejaculation	0.8 ± 0.4	0.4 ± 0.3

Note. No differences were observed between isolated and nonisolated controls ($n = 10$) on the number of mounts, intromissions, and ejaculations during a 20-min test. Data are presented as mean number \pm SEM.

formance on the EPM (Da Silva, Ferreira, Carobrez, & Morato, 1996). A possible explanation for this discrepancy could be the housing condition at the time of testing, i.e., individual versus social housing. Social housing can eliminate the transient effects of isolation such as increased fearfulness (Potegal & Einon, 1989). Isolation-induced hyperactivity and altered performance on the EPM are mostly observed in rats which were individually housed at the time of testing, in contrast to the experiments described in this article where the rats were socially housed at the time of testing. Other factors could account for the observed differences in relation to open-field results, such as time of weaning, time and duration of the isolation period, the duration of the test or light conditions during the test session, and/or which parameters were used to assess locomotor activity. In contrast to the previously mentioned studies, an automated behavioral observation system was used which enabled calculation of the exact traveled distance of the rats in centimeters. Furthermore, EPM can be used as an internal control for general activity by measuring the total number of crossings: It showed similar activity levels of both the isolated and nonisolated control rats.

While no differences were observed in nonsocial situations, clear behavioral differences between isolated and nonisolated controls were demonstrated in social challenges. Deprivation of early social interactions in young rats during a period in which social play is most abundant (Weeks 4 and 5 of age) resulted in a decreased social activity, i.e., less approach behavior and anogenital sniffing as compared to nonisolated controls. As no differences were observed between isolated and nonisolated control rats on open-field activity and EPM performance, the reduction of approach behavior in the social interaction test could not be due to decreased locomotor activity or to increased anxiety. Decreased social activity in juvenile deprived rats is also reported by Meaney and Stewart (1979).

They reared 22-day-old rats to maturity in social isolation while they remained individually housed. Although our rats were socially housed at the time of testing, we found similar results, indicating that depriving male rats of early social experiences permanently alters their pattern of social behavior independent of the housing conditions at the time of testing. It should be noted that increased social activity is sometimes observed after isolation (Hol, Ruven, Van Ree & Spruijt, 1996; Meaney & Stewart, 1979). Differential effects of isolation are reported depending on the age of isolation, time of testing, and the housing condition at the time of testing, which could indicate that there is a sensitive period for the development of normal socio-affective responses in the rat.

The results obtained in the sexual interaction test confirm the decreased social interest in isolated rats. Less anogenital sniffing was observed in juvenile deprived rats, although the capacity to perform sexual motor acts was not affected. Several authors described comparable experiments and reported abnormal patterns of sexual behavior: Interaction with both the mother and littermates seem to be prerequisites for the appearance of normal sexual behavior, i.e., mounting, intromission and ejaculations in adulthood (Gerall, 1963; Gerall et al., 1967; Gruendel & Arnold, 1969; Hård & Larsson, 1968). However, our data show that especially the socio-sexual interactions are disturbed, indicating that the contact with littermates is probably important to establish social bonds between sexual partners. Juvenile isolation also altered the behavioral reactions to territorial aggression induced by social defeat. For instance, isolated rats took significantly longer to assume a submissive posture when attacked by the dominant resident. This resulted in a larger number of attacks (data not shown) as previously reported in studies on the effects of prolonged isolation on intraspecific aggression (Lore & Flannelly, 1977). After the agonistic encounter, juvenile deprived rats hardly showed immobility behavior compared to non-isolated control rats, but exhibited exploration behavior. Isolation-rearing apparently produces an adult rat that shows highly abnormal social behavior, provoking attacks by the resident rats. If territorial aggression can be seen as a social stressor, then isolation-induced changes in responsivity to stressors may be specific for social situations as no differences were observed between the isolated and nonisolated rats in the non-social shock prod test. It should be noted however, that the social aspect is not the only difference in the nature of both stressors.

Confrontation with the dominant resident resulted in increased plasma concentrations of CORT, A, and

NA. Baseline levels of CORT, NA, and A were comparable to earlier reported studies using identical techniques to measure catecholamines and CORT (Scheurink, Steffens & Gaykema, 1990; Sgoifo, de Boer, Haller & Koolhaas, 1996). In previously isolated rats, the increases in plasma CORT and A levels caused by social defeat were significantly potentiated. Comparing the behavioral results with the hormonal data, it seems that juvenile deprivation has not resulted in a diminished impact of the challenge but rather in an inability to select the appropriate behavioral strategy to prevent further attacks. The ineffective behavioral response would then result in increased neuroendocrine reactivity. However, it is unclear whether this is due only to the isolation period early in life. For instance, it could be that rehousing the rats after the isolation period with identically reared rats (in groups of 5 rats) differentially affects the "stress" load of isolated and nonisolated rats.

In the present studies, we deprived young rats of a specific early social experience: "rough-and-tumble" play between 22 and 35 days of age. Social play behavior markedly increases and peaks during this period, and subsequently decreases with age but does not completely disappear (Baenninger, 1967; Meaney & Stewart, 1981; Vanderschuren, Niesink & Van Ree, 1997). Interestingly, during rough-and-tumble play postures can be observed that resemble social, sexual, and agonistic behavioral patterns displayed by adult rats. It should be noted that social isolation after weaning is not only deprivation from social play, but from all forms of social interactions. Long-term isolation from weaning results in more severe disturbances in a variety of behaviors (Da Silva et al., 1996; Hård & Larsson, 1968), probably caused by social isolation in general and not only play deprivation. Einon and co-workers (1978) support the hypothesis that deprivation of social play in rats especially contributes to the observed isolation-induced effects because short daily periods of social play attenuate the effects of isolation-rearing. We have conducted identical experiments by giving rats 30 min play/day, while keeping them isolated the rest of the day (unpublished observations). These rats did not show the isolation-induced effects described in the present article.

In conclusion, for rats social play early in life is necessary for an adequate development of appropriate response patterns in social situations later in life (Einon, Humphreys, Chivers, Field & Naylor, 1981). Isolation early in life does not result in general changes in responsivity because in nonsocial situations no behavioral differences were observed between the experimental groups.

REFERENCES

- Baenninger, L. P. (1967). Comparison of behavioural development in socially isolated and grouped rats. *Animal Behaviour*, 15, 312–323.
- Bekoff, M. (1974). Social play and play-soliciting by infant canids. *American Zoologist*, 14, 323–340.
- Bolles, R. C., & Woods, P. J. (1964). The ontogeny of behaviour in the albino rat. *Animal Behaviour*, 12, 427–441.
- Da Silva, N. L., Ferreira, V. M. M., Carobrez, A. P., & Morato, G. S. (1996). Individual housing from rearing modifies the performance of young rats on the elevated plus-maze apparatus. *Physiology & Behavior*, 60, 1391–1396.
- Dawson, Jr., Kontur, R. P., & Monjan, A. (1984). High-performance liquid chromatographic (HPLC) separation and quantitation of endogenous glucocorticoids after solid-phase extraction from plasma. *Hormone Research*, 20, 89–94.
- Diamant, M., Croiset, G., De Zwart, N., & De Wied, D. (1991). Shock-prod burying test in rats: autonomic and behavioral responses. *Physiology & Behavior*, 50, 23–31.
- Einon, D. F., Humphreys, A. P., Chivers, S. M., Field, S., & Naylor, V. (1981). Isolation has permanent effects upon the behavior of the rat, but not the mouse, gerbil, or guinea pig. *Developmental Psychobiology*, 14, 343–355.
- Einon, D. F., & Morgan, M. J. (1978). Early isolation produces enduring hyperactivity in the rat, but no effect upon spontaneous alternation. *Quarterly Journal of Experimental Psychology*, 30, 151–156.
- Einon, D. F., Morgan, M. J., & Kibbler, C. C. (1978). Brief periods of socialization and later behavior in the rat. *Developmental Psychobiology*, 11, 213–225.
- Gerall, A. A. (1963). An exploratory study of the effect of social isolation variables on the sexual behavior of male guinea pigs. *Animal Behaviour*, 11, 274–282.
- Gerall, H. D., Ward, I. L., & Gerall, A. A. (1967). Disruption of the male rat's sexual behaviour induced by social isolation. *Animal Behaviour*, 15, 54–58.
- Groos, K. (1898). *The play of animals*. New York: D. Appleton.
- Gruendel, A. D., & Arnold, W. L. (1969). Effects of early social deprivation on reproductive behavior of male rats. *Journal of Comparative and Physiological Psychology*, 67, 123–128.
- Hård, E., & Larsson, K. (1968). Dependence of adult mating behavior in male rats on the presence of littermates in infancy. *Brain Behavior and Evolution*, 1, 405–419.
- Hol, T., Ruven, S., Van Ree, J. M., & Spruijt, B. M. (1996). Chronic administration of Org2766 and morphine counteracts isolation-induced increase in social interest: Implication of endogenous opioids. *Neuropeptides*, 30, 283–291.
- Humphreys, A. P., & Einon, D. F. (1981). Play as a reinforcer for maze-learning in juvenile rats. *Animal Behaviour*, 29, 259–270.
- Koolhaas, J. M., Hermann, P. M., Kemperman, C., Bohus, B., Van den Hoofdakker, R. H., & Beersma, D. G. M. (1990). Single social defeat in male rats induces a gradual but long lasting behavioral change: A model for depression? *Neuroscience Research Communications*, 7, 35–41.
- Korte, S. M., Smit, J., Bouws, G. A. H., Koolhaas, J. M., & Bohus, B. (1990). Behavioral and neuroendocrine response to psychosocial stress in male rats: The effects of the 5-HT_{1A} agonist ipsapirone. *Hormones and Behavior*, 24, 554–567.
- Lore, R., & Flannelly, W. (1977). Rat societies. *Scientific American*, 236, 106–118.
- Meaney, M. J., & Stewart, J. (1979). Environmental factors influencing the affiliative behavior of male and female rats (*Rattus norvegicus*). *Animal Learning and Behavior*, 7, 397–405.
- Meaney, M. J., & Stewart, J. (1981). A descriptive study of social development in the rat (*Rattus norvegicus*). *Animal Behaviour*, 29, 34–45.
- Niesink, R. J. M., & Van Ree, J. M. (1989). Involvement of opioid and dopaminergic systems in isolation-induced pinning and social grooming of young rats. *Neuropharmacology*, 28, 411–418.
- Panksepp, J., Siviy, S., & Normansell, L. (1984). The psychobiology of play: Theoretical and methodological perspectives. *Neuroscience and Biobehavioral Reviews*, 8, 465–492.
- Poole, T. B., & Fish, J. (1976). An investigation of individual, age, and sexual differences in the play of *Rattus norvegicus* (Mammalia: Rodentia). *Journal of Zoology of London*, 179, 249–260.
- Potegal, M., & Einon, D. (1989). Aggressive behavior in adult rats deprived of playfighting experience as juveniles. *Developmental Psychobiology*, 22, 159–172.
- Robbins, T. W., Jones, G. H., & Wilkinson, L. S. (1996). Behavioral and neurochemical effects of early social deprivation in the rat. *Journal of Psychopharmacology*, 10, 39–47.
- Scheurink, A. J. W., Steffens, A. B., & Gaykema, P. A. (1990). Hypothalamic adrenoreceptors mediate sympathoadrenal activity in exercising rats. *American Journal of Physiology*, 259, 470–477.
- Scott, J. P., & Marston, M. V. (1950). Critical periods affecting the development of normal and maladjustive social behavior of puppies. *The Journal of Genetic Psychology*, 77, 25–60.
- Sgoifo, A., de Boer, S. F., Haller, J., & Koolhaas, J. M. (1996). Individual differences in plasma catecholamines and corticosterone stress responses of wild-type rats: Relationship with aggression. *Physiology & Behavior*, 60, 1403–1407.
- Small, W. S. (1899). Notes on the psychic development of the young white rat. *American Journal of Psychology*, 11, 80–100.
- Smedes, F., Kraak, J. C., & Poppe, H. (1982). Simple and fast solvent extraction system for selective and quantitative isolation of adrenaline, noradrenaline, and dopamine from plasma and urine. *Journal of Chromatography*, 231, 25–39.

- Spruijt, B. M., Josephy, M., Van Rijzingen, I., & Maaswinkel, H. (1994). The ACTH (4-9) analog Org2766 modulates the behavioral changes induced by NMDA and the NMDA receptor antagonist AP5. *Journal of Neuroscience*, 14, 3225–3230.
- Steffens, A. B. (1969). A method for frequent sampling of blood and continuous infusion of fluids in the rat without disturbing the animal. *Physiology & Behavior*, 4, 833–836.
- Taylor, G. T. (1980). Fighting in juvenile rats and the ontogeny of agonistic behavior. *Journal of Comparative and Physiological Psychology*, 94, 953–961.
- Vanderschuren, L. J. M. J., Niesink, R. J. M., & Van Ree, J. M. (1997). The neurobiology of social play behavior in rats. *Neuroscience and Biobehavioral Reviews*, 21, 309–326.
- Vanderschuren, L. J. M. J., Stein, E. A., Wiegant, V. M., & Van Ree, J. M. (1995). Social isolation and social interaction alter regional brain opioid receptor binding in rats. *European Neuropsychopharmacology*, 5, 119–127.